

Attorney Docket No.: UMD-0111
Inventors: Jeffrey B. Kaplan
Serial No.: Not yet assigned
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Listing of the claims:

Claim 1 (original): An isolated nucleic acid sequence encoding soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof which promotes detachment of bacterial or fungal cells from a biofilm.

Claim 2 (currently amended): The isolated nucleic acid sequence of claim 1 comprising a nucleic acid sequence with 50% sequence identity to at least 30 contiguous nucleotides of SEQ ID NO:1, 3, 5, 7 or 9.

Claim 3 (currently amended): The isolated nucleic acid sequence of claim 1 comprising a nucleic acid sequence of SEQ ID NO:1, 3, 5, 7 or 9.

Claim 4 (currently amended): A nucleic acid sequence encoding a fusion polypeptide comprising the isolated nucleic acid sequence of claim 1, ~~2 or 3~~ and a second nucleic acid sequence encoding a second polypeptide.

Claim 5 (original): A vector comprising the nucleic acid sequence of claim 1, 2, 3 or 4.

Claim 6 (original): A host cell comprising the vector of claim 5.

Claim 7 (original): An isolated amino acid sequence encoded by the nucleic acid sequence of claim 1, 2, 3 or 4.

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Claim 8 (original): An isolated soluble, β -N-acetylglucosaminidase protein or active fragment or variant thereof which promotes detachment of bacterial or fungal cells from a biofilm.

Claim 9 (original): The isolated soluble, β -N-acetylglucosaminidase protein or active fragment or variant thereof of claim 8 comprising SEQ ID NO:2, 4, 6, 8 or 10.

Claim 10 (original): A fusion protein comprising the amino acid sequence of claim 8 or 9 and a second polypeptide.

Claim 11 (original): A pharmaceutical composition comprising an effective amount of the isolated soluble, β -N-acetylglucosaminidase protein or active fragment or variant thereof of claim 8 or 9 and a pharmaceutically acceptable carrier.

Claim 12 (original): A method for enhancing efficacy of an antibiotic against a bacterial infection comprising administering the pharmaceutical composition of claim 11 in combination with or prior to administration of the antibiotic.

Claim 13 (original): A medical device coated with the isolated soluble, β -N-acetylglucosaminidase protein or active fragment or variant thereof of claim 8 or 9.

Claim 14 (original): A wound healing device impregnated with the isolated soluble, β -N-acetylglucosaminidase protein or active fragment or variant thereof of claim 8 or 9.

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Claim 15 (original): A liquid antiseptic solution comprising the isolated soluble, β -N-acetylglucosaminidase protein or active fragment or variant thereof of claim 8 or 9.

Claim 16 (original): A method for inhibiting detachment of bacterial or fungal cells from biofilm comprising mutating a *dspB* gene of bacterial cells to inhibit detachment of bacterial or fungal cells from biofilms.

Claim 17 (original): A method for inhibiting detachment of bacterial or fungal cells from biofilm comprising decreasing expression or levels of soluble, β -N-acetylglucosaminidase or inhibiting activity of soluble, β -N-acetylglucosaminidase in the bacterial cells so that detachment of bacterial or fungal cells from the biofilm is decreased.

Claim 18 (original): An isolated mutant of *Actinobacillus actinomycetemcomitans* which forms biofilm colonies which tightly adhere to surfaces but which are unable to release cells into the medium or spread over the surface.

Claim 19 (original): The mutant of claim 18 wherein the *dspB* gene is mutated.

Claim 20 (original): A method for identifying an agent which modulates detachment of bacterial or fungal cells from biofilms comprising assessing an agent's ability to modulate activity or expression or levels of soluble, β -N-acetylglucosaminidase.

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Claim 21 (original): A method for promoting detachment of bacterial or fungal cells from a biofilm comprising contacting bacterial cells with soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof or a nucleic acid sequence encoding soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof so that detachment of bacterial or fungal cells from a biofilm is promoted.

Claim 22 (original): A method for reducing risk of infection of an organism by bacteria or fungi on a medical device or surgical instrument comprising contacting the medical device or surgical instrument with soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof prior to contacting the organism with the medical device or surgical instrument.

Claim 23 (original): The method of claim 22 wherein the medical device is coated with the soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof.

Claim 24 (original): The method of claim 23 wherein the coating of soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof is dried on the medical device.

Claim 25 (original): The method of claim 22 wherein the medical device is a catheter and the soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof is in a catheter lock solution in the catheter.

Claim 26 (original): A method for inhibiting, preventing or treating bacterial or fungal infections comprising

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administering to an organism soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof or a nucleic acid sequence encoding soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof so that detachment of bacterial or fungal cells from a biofilm is promoted.

Claim 27 (original): The method of claim 26 wherein the bacterial or fungal infection is from a bacterium or fungus that produces a N-acetylglucosaminidase containing biofilm polysaccharide that can be degraded by soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof.

Claim 28 (original): The method of claim 26 wherein the soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof or a nucleic acid sequence encoding soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof is administered as a coating on a medical device implanted in the organism.

Claim 29 (original): The method of claim 26 wherein the soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof or a nucleic acid sequence encoding soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof is administered as a pharmaceutical composition.

Claim 30 (original): The method of claim 26 wherein the soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof or a nucleic acid sequence encoding soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof is incorporated into a liquid disinfecting solution and applied

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topically to the subject prior to insertion of an implantable medical device.

Claim 31 (original): The method of claim 26 wherein a wound dressing applied to the subject is impregnated with the soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof or a nucleic acid sequence encoding soluble, β -N-acetylglucosaminidase or an active fragment or variant thereof.

Claim 32 (original): A primer pair which identifies bacteria with *DspB* homologs.

Claim 33 (original): The primer pair of claim 32 comprising SEQ ID NO:12 and SEQ ID NO:13.

Claim 34 (original): A kit for identifying bacteria with *DspB* homologs comprising the primer pair of claim 32 or 33 and instructions for use of the primer pair to identify bacteria with *DspB* homologs.